EXHIBIT RAS-5

This is exhibit RAS-5 referred to in Declaration Under 37 C.F.R. 1.132 by Richard Anthony Strugnell dated 27.9.01

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Richard Strugnell

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10-11-01

IN THE MATTER OF U.S. Patent Appl. No. 09/425,956 in the name of Tanzi *et al.*

DECLARATION UNDER 37 C.F.R. 1.132

I, Richard Anthony Strugnell, hereby declare as follows:

1. My scientific training and experience are as documented on the attached abridged curriculum vitae. A copy of this document is provided herewith as Exhibit RAS-1.

2. I am currently employed as an Associate Professor at the Department of Microbiology and Immunology of the University of Melbourne ("my institution"), located in Melbourne, Victoria. My recent past employment history is also documented on the attached abridged curriculum vitae.

3. Prior to and by 19 October 1994, I was (and currently am) experienced in producing both monoclonal and polyclonal antibodies for a variety of uses. I have been producing monoclonal antibodies since the early 1980's and polyclonal antisera since 1979. Consequently, I am familiar with the potential applications and limitations of both techniques.

4. I have read and considered United States Patent Appl. No. 09/425,956, entitled "A Diagnostic Assay for Alzheimer's Disease" ("the '956 application"), which has a priority date of 19 October 1994. I am not an inventor of this patent application.

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5. In addition, I have read and considered the journal article by Iwatsubo *et al.* (published July 1994), "Visualization of Aβ42(43) and Aβ40 in senile plaques with end-specific Aβ Monoclonals: Evidence that an initially deposited species is Aβ42(43)." Neuron **13**:45-53. A copy of Iwatsubo *et al* is provided herewith as Exhibit RAS-2.

6. I have also read and considered the Office Action dated 3 April 2001 for the '956 application. My comments are in relation to the claim rejections made by the Examiner under **35 U.S.C. § 112** in paragraph 11 of the Office Action.

7. It is the Examiner's position that the specification of the '956 application does not provide sufficient detail to teach a person of ordinary skill in the art to make or use the invention recited in claims 5 to 16. Claims 5 to 16 are directed to a diagnostic assay which requires polyclonal antisera that specifically bind to one of the two peptides, amyloid β_{1-40} and amyloid β_{1-42}, without cross-reacting with the other. The Examiner contends that the generation of polyclonal antibodies is unpredictable and difficult to reproduce, citing Campbell, A.M., "Monoclonal Antibody and Immunosensor Technology," in *Laboratory Techniques in Biochemistry and Molecular Biology*, Vol. 23, van der Vliet, P.C., ed., Elsevier, Amsterdam, The Netherlands, pp. 1-6 (1991). The Examiner also states that a person of ordinary skill in the art would not know which immunogens to use or how to screen for antibodies with the claimed characteristics

8. I respectfully disagree with the Examiner. I submit that prior to and at the priority date (19 October 1994) of the '956 application, a person of ordinary skill in the art of preparation of monoclonal and polyclonal antibodies could have readily generated specific polyclonal antibodies to each of amyloid β_{1-40} and amyloid β_{1-42}, merely by using routine techniques that were available prior to and at the priority date, to practice the claimed invention.

9. After considering Iwatsubo *et al.*, at the priority date a person of ordinary skill in the preparation of monoclonal and polyclonal antibodies would have recognised that there must be unique epitopes on both amyloid β_{1-40} and amyloid β_{1-42} which enabled the production of specific monoclonal antibodies against each of these peptides. Consequently, a person of ordinary skill would have recognised that in order to produce a polyclonal antisera specific for each peptide, it would be necessary to select an appropriate immunogen bearing these respective unique epitopes.

10. In scientific research it is commonly required to generate antibodies that are capable of distinguishing between two or more similar molecules. As of the priority date, it was well known in the art that the smallest linear epitope in a peptide that was capable of being recognised by an antibody was as small as two and three amino acids in length. Geysen *et al.* (Proc Natl Acad Sci U S A 1984 81:3998-4002) showed as early as 1984 that peptide epitopes could critically depend on one or two amino acids, when they

performed substitution studies to show defined the binding specificity of antibodies. A copy of this citation is provided herewith as Exhibit RAS-3.

11. Studies reported in 1988 showed that the minimal peptide epitopes could be defined by overlapping peptides, using a process termed "Pepscan". In this initial Pepscan study, the V3 loop of HIV gp120 was found to contained epitopes of 5 amino acids or less (AIDS 1988 2:157-64). A copy of this citation is provided herewith as Exhibit RAS-4. Linear B cell epitopes were further minimalised in size once the Pepscan technique became more widely available in the late 1980s, and early 1990s (eg. J Gen Virol 71:881-7). A copy of this citation is provided herewith as Exhibit RAS-5. Subsequent studies by Xing *et al* (Mol Immunol 1992 29:641-50) showed that peptide epitopes as short as 3-4 amino acids could be resolved by overlapping peptides and that some of these small epitopes could act as immunogens (ie. elicit antibodies) if presented in the appropriate context. A copy of Xing et al (1992) is provided herewith as Exhibit RAS-6.

12. A person of ordinary skill in the art would have recognised that by determining which epitopes Iwatsubo *et al.*'s antibodies bound, he/she could generate synthetic immunogens that would enable production of specific polyclonal antisera. The amino acid sequences of both amyloid $\beta_{1\text{-}40}$ and amyloid $\beta_{1\text{-}42}$ were also known at the priority date. As the two sequences differed only by two amino acid residues at the carboxyl terminus, a person of ordinary skill in the art would have known that it would be the carboxy end of these molecules that would bear the respective unique epitopes.

13. In order to determine which linear amino acid sequences provided the unique epitopes at the carboxyl terminus of amyloid $\beta_{1\text{-}40}$ and amyloid $\beta_{1\text{-}42}$ that were recognised by Iwatsubo *et al.*'s monoclonal antibodies, the person of ordinary skill in the art would have generated a range of small synthetic peptides from the carboxyl end of these molecules using the "Pepscan" technique. The Pepscan technique, which is also known as "General Net Analysis" or "Multipin Epitope Mapping", relies on the chemical synthesis of short (6-10 residues) overlapping peptides on plastic pins. The technique was available as an international commercial service through several companies, including Coselco Mimotopes (11 Duerdin St. Clayton, VIC, AUSTRALIA) from the late 1980's onwards.

14. Using routine assays such as dot blots or ELISA, the person of ordinary skill in the art would have then probed these small synthetic peptides with Iwatsubo *et al.'s* monoclonal antibodies, or other amyloid β-specific monoclonal antibodies generated using the techniques disclosed by Iwatsubo *et al.* By determining which peptides were recognised by these specific antibodies, the person of ordinary skill would have determined the amino acid sequences that provide the unique epitopes recognised by the specific antibodies. These sequences would have been conjugated to carrier proteins using standard techniques and used as immunogens to generate specific polyclonal antibodies.

15. Prior to the invention of monoclonal antibodies by Kohler and Milstein in 1975, polyclonal antibodies served as fundamental reagents for the analysis of proteins, carbohydrates and lipids. The advent of monoclonal antibodies, while very important, did not obviate the need for polyclonal antibodies. Polyclonal antibodies were, and still are, elicited in many animal species (usually rabbits) by the simple administration of antigen and adjuvant. This straightforward process was, and still is, very reliable and has been used to generate, for example, the typing reagents used to classify more than 2000 serologically-identifiable variants of *Salmonella enterica.* There are numerous publications prior to 1994 which outline methods for improving the specificity and affinity of polyclonal antisera production, for example binding antigen to nitrocellulose (Anal Biochem. 1987 163:136-42), immunization with precipitin lines (Methods Enzymol. 1981 73:52-7), or multiple intradermal inoculations with small amounts of antigen (Methods Enzymol. 1981 73:46-52). A copy of these citations are provided herewith as Exhibits RAS-7, RAS-8 and RAS-9 respectively.

16. Polyclonal typing sera remain very important reagents in many elements of both routine diagnostic and experimental serology. The reproducibility of producing polyclonal antibodies is dependent on a number of factors, but these factors are controllable. They include the method of antigen preparation, the type of adjuvant used (eg. Alum- or oil-based), and the schedule and route of immunisation. If care is taken over the preparation of antigen in adjuvant, and the immunisations are performed by someone who could perform the basic inoculations (intramuscular or subcutaneous), then the serological responses in immunised animals eg. rabbits were and are generally reproducible. Undergraduate (B.Sc) practical classes in Immunology at Monash University, Melbourne Victoria taught, in 1978 and earlier,

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the theoretical and practical aspects of raising polyclonal sera, including tests for stability of the adjuvant mixture, the location of appropriate muscles or subcutaneous sites for injection, and schedules which lead to development of good serological responses. These and very similar procedures were used by Companies such as Wellcome Diagnostics to generate polyclonal typing antisera and are still used today by Asla Ltd. <http://www.asla-biotech.com/asla-ab.htm> and others.

17. As synthetic forms of both amyloid β_{1-40} and amyloid β_{1-42} peptides were commercially available at this time, it would have been routine for one of ordinary skill in the art to determine whether the polyclonal antibodies generated with this technique were specific.

18. Antibody binding assays could be performed using either liquid or solid phase approaches based on peptides synthesized with or without the carboxyl terminus of amyloid β_{1-42}. ELISA assays were available from the early 1980s onwards, other solid phase assays ranging from Dot Blots through simple gel diffusion assays were also used to identify antigen/antibody reactions. More importantly, overlapping peptides had been used to define the fine specificity of polyclonal antibodies since at least 1990 (for example Infect Immun. 1990 58:1450-5). A copy of this citation is provided herewith as Exhibit RAS-10. Moreover, small synthetic peptides were used in competitive liquid phase assays to block antibody binding, and to prove the binding specificity of monoclonal antibodies (Mol Immunol. 1992 29:641-50). A copy of this citation is provided herewith as Exhibit RAS-11. Synthetic peptides could similarly have been used to show binding specificity of polyclonal antibodies raised against synthetic peptides comprising the carboxyl terminus of amyloid β_{1-42}.

19. Consequently, I respectfully submit that at the priority date of the '956 application, the identification and synthesis of immunogens, and the generation and identification of polyclonal antisera specific to amyloid β1-40 or amyloid β1-42 using the immunogens only required routine experimentation by a person of ordinary skill in the art, not undue experimentation. Accordingly, the Examiner's rejection should be withdrawn.

20. I have read and understood 37 C.F.R. 10.18(b) and (c). I declare under penalty of perjury of the laws of the United States that all statements made herein of my own personal knowledge are true and correct, that all statements made upon information

and belief are believed to be true and correct, and I will testify to same if called to do so.

Date: 27.9.01

Signature: _____
Richard Anthony Strugnell